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Growth and fatty acid profile of the marine microalga *Picochlorum* Sp. grown under nutrient stress conditions

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KEYWORDS

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Abstract Growth, proteins, carbohydrates and chlorophyll a as well as fatty acid compositions of a native marine microalga *Picochlorum* Sp. were studied in batch culture at light intensity 100 μ mol photons m^{-2} s^{-1}, temperature 25 ± 1 °C and 16:8 h light and dark diurnal cycles using Walen medium. Under nutrient stresses, the cell counts and biomass productivity of the tested alga decreased as compared by control culture after 12 days. Carbohydrate content increased by nearly 21%, and 44% in the cultures grown in media supplemented with (−50% & −100% $NaNO_3$), respectively. The proteins showed a remarkable decrease by 54% and 69.7% under the same conditions, respectively. chl *a* contents of *Pichochlorum* Sp. culture grown under N-starvation (−100%) decreased and also, a yellowish colour was recorded in the culture. The lipids increased by about 0.55, 1.6 folds in the cultures growing on the medium containing (−50% & −100% $NaNO_3$), respectively and a decrease by 0.6% in those growing on the media containing nitrogen (+100% $NaNO_3$), respectively. Considering phosphorus stress, the carbohydrate content increased by nearly 30.27%, 62.38% for the culture growing on −50% and −100% $NaH_2PO_4{\cdot}2H_2O$ with respect to the control culture. The proteins showed a remarkable decrease by 24.5%, 37.3% in the culture medium containing the same concentrations, respectively. On the other hand, phosphorus deficiency (−50%) caused an increase in the chl *a* level of the cultures. Similarly, the lipids increased by about 2.2% and 2.5 folds in the cultures growing on the phosphorus deficient media (−50% & −100%), respectively. Fatty Acid Methyl Ester (FAME) was also found to be improved in algal cultures grown under nitrogen & phosphorus stress (−50% & −100%) which are mainly saturated fatty acids. The unsaturation of the FAME profile is crucial for the overall performance of the final produced biofuel. With further augmentations of lipid & carbohydrate content and improved fatty

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acids, the native microalga strain could be a potent candidate for aqua-culture feeding and or biofuel production.

Introduction

Microalgae biomass contains products with high commercial importance like proteins, lipids, carbohydrates (Torzillo and Vonshak, 2004).

Nutrient availability has a significant impact on growth and propagation of microalgae and broad effects on their lipid and fatty acid composition. Environmental stress condition when nutrients are limited, invariably causes a steadily declining cell division rate. Surprisingly, active biosynthesis of fatty acids is maintained in some algae species under such conditions, provided there is enough light and CO_2 available for photosynthesis (Thompson, 1996).

Nitrogen and phosphate are two important macronutrients for growth and metabolism of algal cells. Nitrogen is a fundamental element for the formation of proteins and nucleic acids. Being an integral part of essential molecules such as ATP, the energy carrier in cells, phosphate is another very important nutrient. Phosphate is also a part of the backbone of DNA and RNA, which are essential macromolecules for all living cells. Phosphorus is also a key component of phospholipids. It is not unusual for algae to become nutrient-limited (*i.e.*, nitrogen- and phosphorus-limited) in the natural environment (Harris, 1986).

One who has grown microalgae under laboratory or outdoor condition is well aware of the fact that to obtain high lipid content, external stress or lipid induction techniques need to be applied. Many microalgae produce saturated and unsaturated fatty acids naturally under ideal growth conditions, which have high nutritional value, but are less ideal for biofuels. However, the synthesis of neutral lipids in the form of Triacylglycerides (TAGs) can be induced in many species under stress conditions. (Miao and Wu, 2006; Hu et al., 2008). There has been a wide range of studies carried out on lipid induction techniques in microalgae such as the use of nutrients stress, including nitrogen and/or phosphorus starvation (Juneja et al., 2013). Due to the high growth rates and ease of cultivation Chlorophyta is considered to be a promising phylum for biofuel production.

The green alga Picochlorum Sp. was considered previously as promising feed stocks for large scale production of biofuels (De la Vega et al., 2011). The unsaturation of the FAME profile is crucial for the overall performance of the final produced biofuel. For instance, biodiesel is mainly constituted of SFA and MUFA, since PUFA decrease the final stability of biodiesel. Furthermore, the fatty acid methyl ester profile of Picochlorum Sp. seems ideal for biodiesel production due to a low degree of polyunsaturated fatty acid methyl esters and high amount of palmitic and oleic acids (Demirbas, 2009; Pereira et al., 2013).

Therefore, the main objectives of this study were: (i) monitoring the growth of Picochlorum Sp. under normal conditions and nutrient stress ones i.e. nitrogen and Phosphorus, (ii) estimation of cellular contents of proteins, carbohydrates, chlorophyll and lipid as well as fatty acid profile in the algal species under nitrogen limitation and phosphorus limitations.

Materials and methods

The alga strain and growth conditions

The alga strain *Picochlorum* Sp. was obtained from Southern Company for Fish Farming, Djerba, Tunisia. It was cultivated axenically as batch cultures in 1 L Erlenmeyer flasks with Walens medium (Walne, 1970) at an initial counts of 10×10^6 colony ml^{-1}. For the production of biomass, exponentially growing algae culture was transferred into fresh sterile medium [10% (v/v) of inoculums]. Cultures were illuminated by tubular fluorescent lamps (PHILIPS Master TL-D 85 W/840). The light intensity at the surface of the culturing vessels was 100 μ mol photons m^{-2} s^{-1} with a photoperiod of 16:8 h light: dark at 25 ± 1 °C.

The effect of different nutrients namely $NaNO_3$ [(control (20.0 g L^{-1}), −100% (0 g L^{-1}), −50% (10 g L^{-1}) and +100% (40 L^{-1})], $NaH_2PO4{\cdot}2H_2O$ [(control (100 g L^{-1}), −100% (0 g L^{-1}), −50% (50 g L^{-1}) and + 100% (200 g L^{-1}), on growth and biochemical composition of *Picochlorum* Sp. were studied.

Monitoring of algal growth

The growth of alga was monitored by determining the cell density using a haemocytometer slide, where cell numbers were estimated at 48 h intervals in addition to the determination of algal cellular dry weight (CDW) and biomass productivity that was calculated as according to Abomohra et al. (2012).

Biomass productivity g CDW L^{-1} d^{-1} = ($CDW_L - CDW_E$)·(t_L _ t_E) with CDW_E represents the CDW (g L^{-1}) at days of early exponential phase (t_E) and CDW_L at days of late exponential phase (t_L). Biomass was determined as cellular dry weight (CDW) and measured gravimetrically. A known volume of culture was filtered through pre-weighed and pre-combusted GF/C filter paper. The filtered cell mass was oven dried at 80 °C for 6 h until constant weight, cooled down to room temperature in desiccators and measured the dry weight of the sample using an analytical balance with a precision of 0.1 mg. Biomass was expressed in grams per litre (g L^{-1}).

Biochemical composition of Picochlorum Sp.

Total protein was extracted from the algal cells, according to the method of Rauch, 1981. Protein content, both total and water-soluble, was determined according to Hatree (1972). Chlorophyll a (chl *a*) content of an algae was determined spectrophotometrically after extraction with acetone (Parsons and Strickland, 1963). Carbohydrate content was estimated according to the methods of Dubois et al. (1956). The total

lipid was extracted according to Bligh and Dyer (1959). As such, all measurements were carried out in triplicate. Preparation of fatty acid methyl ester from total lipid was performed according to Radwan (1978). All analyses for identification of fatty acid fractions were performed using gas chromatography (GC system Hp, Germany, serial No 6890 D 1530 A serial DE 00000348) equipped with a flame ionization detector; the packing column material was SP-2340.

Results

Influence of nitrogen concentration on Picochlorum Sp. growth

Nutrient availability has a significant impact on growth and propagation of microalgae and broad effects on their lipid and FA composition. Nitrogen is the most growth-limiting factor for eukaryotic microalgae and would be one of the first nutrients to be depleted during algae cultivation. It is relatively easy to apply controlled nitrogen stress on microalgae by subtracting the nitrogen source in the growth media. The effect of different concentrations of $NaNO_3$ on the growth of *Picochlorum* Sp. was recorded as cell counts (cell number ml^{-1}) every other day and by determination of algal cellular dry weight (CDW) for 12 days (Fig. 1).

Picochlorum Sp. was grown on the medium supplemented with different concentrations of $NaNO_3$ (−50%, +100%, −100%) in addition to the control medium. The tested algal species was found to grow in all the four concentrations of $NaNO_3$. The algal growth was greatly affected by the concentration of $NaNO_3$. In the culture medium containing only −50% of ($NaNO_3$), *Picochlorum* Sp. growth was inhibited by nearly the third with respect to the control. The most pronounced inhibition amounting to 51% below the control culture was recorded at −100% of $NaNO_3$. Similarly, biomass productivity (Table 1), was negatively influenced on growing the tested algal species on the medium supplemented with −50% and +100% as well as that grown on −100% $NaNO_3$.

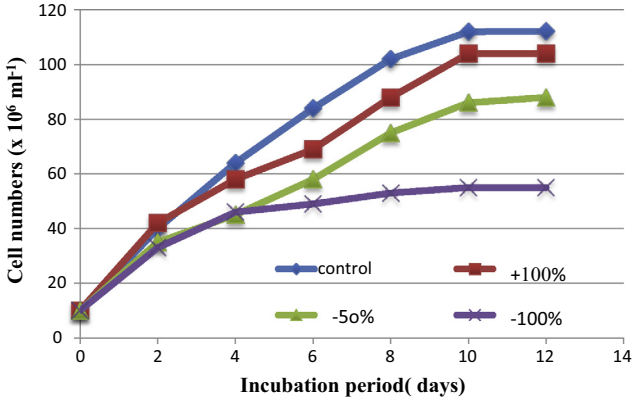

Figure 1 Effect of different concentrations of $NaNO_3$ on the cell number of *Pichochlorum* Sp. during 12 days of incubation.

Influence of phosphorus concentration on Picochlorum Sp. growth

Considering the effect of different phosphorus concentrations ($NaH_2PO_4{\cdot}2H_2O$), the growth of *Picochlorum* Sp. was recorded as cell number ml^{-1} every other day and by determination of algal cellular dry weight (CDW) for 12 days. *Picochlorum* Sp. was grown on the medium containing −50%, +100% and −100% of $NaH_2PO_4{\cdot}2H_2O$ with respect to the control. The results represented in Fig. 2 showed that the tested algal species was found to grow in all the four concentrations. On the decrease or increase of $NaH_2PO_4{\cdot}2H_2O$, the growth was retarded. In the culture medium containing only (−50% $NaH_2PO_4{\cdot}2H_2O$), the algal growth was inhibited by nearly 35% with respect to the control. The most pronounced inhibition amounted to 45.4% below the control was recorded at −100% of $NaH_2PO_4{\cdot}2H_2O$. Biomass productivity was negatively influenced on growing the tested algal species on the medium supplemented with −50% and −100% $NaH_2PO_4{\cdot}2H_2O$. It decreased by nearly 30%, 57%, respectively as compared by control culture (Table 1) after 12 days.

Influence of different concentrations of nitrogen on the Picochlorum Sp. cellular contents

The results (Table 1) revealed that the carbohydrate content exhibits the same pattern, where it was decreased by 30%, for the culture growing on +100% $NaNO_3$ and surprisingly increased by nearly 21%, and 44% in the cultures grown in media supplemented with (−50% & −100% $NaNO_3$), respectively.

In contrast to the carbohydrate content of *Picochlorum* Sp. the proteins showed a remarkable decrease by 54% and 69.7% for the algal cultures growing on media containing −50% and −100% nitrogen medium, respectively. In this study, it was observed that chl *a* contents of *Pichochlorum* Sp. culture grown under N-starvation (−100%) decreased and also, a yellowish

Table 1 Effect of different concentrations of $NaNO_3$ & $NaH_2PO_4{\cdot}2H_2O$ on the biochemical composition and fatty acid (FA) content of *Pichochlorum* Sp. during 12 days of incubation.

Treatment	Element concentrations	Biomass productivity (g CDW L^{-1} d^{-1})	Carbohyrate content (μg ml^{-1} culture)	Protein (μg ml^{-1} culture)	Chlorophyll (μgl^{-1})	Lipid content (μg ml^{-1} culture)	Conc of FA in (% weight)
	Control	0.160 ± 0.04	10.9 ± 0.3	13.56 ± 0.6	98.43	22.4 ± 0.3	8.61
Nitrogen	+100%	0.153 ± 0.02	7.7 ± 0.4	13.33 ± 0.4	154.90	21.1 ± 0.5	10.43
	−50%	0.147 ± 0.02	13.2 ± 0.5	6.25 ± 0.5	77.11	34.8 ± 0.1	13.22
	−100%	0.68 ± 0.03	15 .7 ± 0.6	4.12 ± 0.2	10.22	58.4. ± 0.2	19.69
Phosphorus	+100%	0.129 ± 0.03	8.7 ± 0.4	12.2 ± 0.2	66.4	19.8 ± 0.2	10.22
	−50%	0.112 ± 0.05	14.2 ± 0.5	10.1 ± 0.5	170.2	50.2 ± 0.7	11.1
	−100%	0. 069 ± 0.04	17 .7 ± 0.6	8.5 ± 0.4	7.9	56.0 ± 0.9	12.30

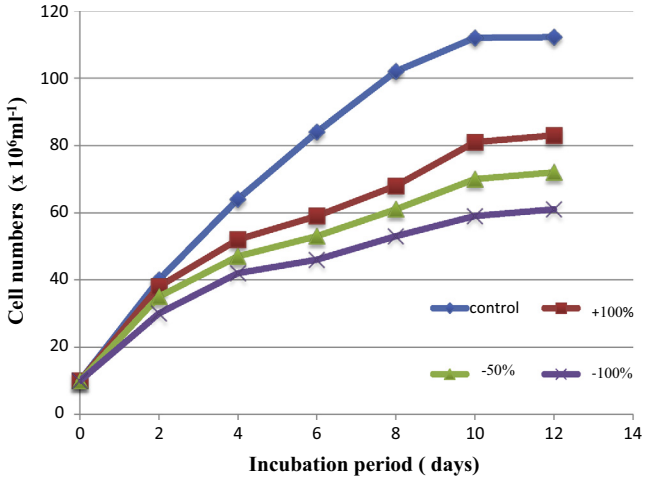

Figure 2 Effect of different concentrations of $NaH_2PO_4{\cdot}2H_2O$ on the cell number of *Pichochlorum* Sp. during 12 days of incubation.

colour was recorded in the culture. The lipids increased by about 0.55, 1.6 folds in the cultures growing on the medium containing (−50% & −100% $NaNO_3$), respectively and a decrease by 0.6% in those growing on the media containing nitrogen (+100%), respectively.

Influence of different concentrations of phosphorus on the Picochlorum Sp. cellular contents

The carbohydrate content exhibited the reverse patterns, where it increased by nearly 30.27%, and 62.38% for the culture growing on −50% and −100% $NaH_2PO_4{\cdot}2H_2O$ with respect to the control culture. The proteins showed a remarkable decrease by 24.5%, and 37.3% in the culture media containing only −50% and −100% $NaH_2PO_4{\cdot}2H_2O$, respectively. On the other hand, Phosphorus-starvation (−50%) caused an increase in the chl *a* level of the cultures. Similarly, the lipids increased by about 2.2% and 2.5 folds in the cultures growing on the phosphorus deficient media (−50% & −100% $NaH_2PO_4{\cdot}2H_2O$), respectively.

Influence of different concentrations of $NaNO_3$ & $NaH_2PO_4{\cdot}2H_2O$ on fatty acid methyl ester (FAME) of Pichochlorum Sp.

As recorded in Table 1 and Fig. 3, fatty acid methyl ester (FAME) was improved in algal cultures grown under nitrogen stress (−50% & −100%). The results in Table 2 showed the individual fatty acids presented in algal culture grown under starvation conditions. The percentage of saturated fatty acids increased by 2 folds (SFA = 14.2) in culture grown under stress medium (−100% $NaNO_3$) compared to the control (SFA = 7.07%). However, polyunsaturated fatty acid (PUFA = 4.92%) was significantly higher in culture grown under the same stress condition. In the algal culture grown under (−100% N), Butyric Acid (C4:0), Caproic Acid (C 6:0), Caprylic Acid (C8:0) increased to be 1.5%, 3.9% and 22.0%, respectively. The most pronounced increase was noticed in Erucic Acid (C22:1) content which increased from 0.33% to 6.50% in the algal culture grown under the same conditions.

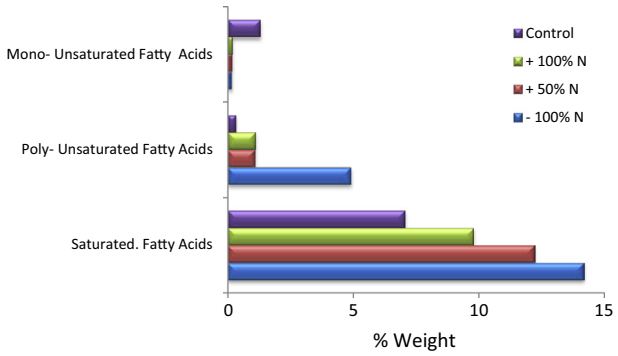

Figure 3 Saturated and unsaturated fatty acid content of *Pichochloruim* Sp. as affected by different concentrations of $NaNO_3$ during 12 days of incubation.

Table 2 Fatty acid methyl ester profile of *Pichochloruim* Sp. grown under nitrogen & phosphorus starvation during 12 days of incubation.

Fatty acid (FA)	Control % FA	-Nitrogen % FA	-Phosphorus % FA
Butyric acid (C4:0)	0.47	1.5	–
Caproic acid (C 6:0)	0.71	3.9	–
Caprylic acid (C8:0)	18.4	22.0	0.09
Capric acid (C10:0)	0.47	0.08	0.04
Undecanoic acid (C11:0)	0.05	0.03	–
Lauric acid (C12:0)	0.49	0.2	1.50
Tridecanoic acid (C13:0)	1.82	0.14	7.62
Myristoleic acid (C14:1)	1.02	0.33	4.90
Myristic acid (C14:0)	1.68	0.32	4.26
cis-10-Pentadecenoic acid (C15:1)	1.13	0.37	5.55
Pentadecanoic acid (C15:0)	0.96	0.31	4.40
Palmitoleic acid (C16:1)	1.38	0.24	2.31
Palmitic acid (C16:0)	3.01	0.63	3.70
cis-10-Heptadecenoic acid (C17:1)	0.16	0.06	0.25
β-Linolenic acid (C18:3)	0.08	0.03	–
Oleic acid (C18:1c)	1.58	0.43	–
Stearic acid (C18:0)	0.34	0.14	0.62
Arachidonic acid (C20:4)	0.60	0.22	1.09
Heneicosanoic acid (C21:0)	0.072	0.03	0.23
Erucic acid (C22:1)	0.33	6.50	9.54
Docosahexaenoic acid – (C22:6)	0.15	–	–

The results in Table 1 and Fig. 4, showed the fatty acid methyl ester (FAME) in algal cultures grown under $NaH_2PO_4{\cdot}2H_2O$ stress (−50% & −100%). The saturated fatty acids decreased (SFA = 6.3%) in culture grown under stress medium (−100% $NaH_2PO_4{\cdot}2H_2O$) compared to the control (the SFA = 7.07%). However, the Monounsaturated fatty acid (MUFA = 5.79%) was significantly higher in culture grown under the same stress conditions.

The results in Table 2 showed the individual fatty acids presented in algal culture grown under (−100% $NaH_2PO_4{\cdot}2H_2O$), Lauric Acid (C12:0), Tridecanoic Acid (C13:0), Myristoleic Acid (C14:1), Myristic Acid (C14:0), cis-10-Pentadecenoic Acid (C15:1), Pentadecanoic Acid (C15:0), Palmitoleic Acid (C16:1) and Erucic Acid (C22:1) were improved and recorded 1.50%, 7.62%, 4.90%, 4.26%, 5.55%, 4.40%, 2.31% and 9.54%, respectively. In contrasts to that present in the control

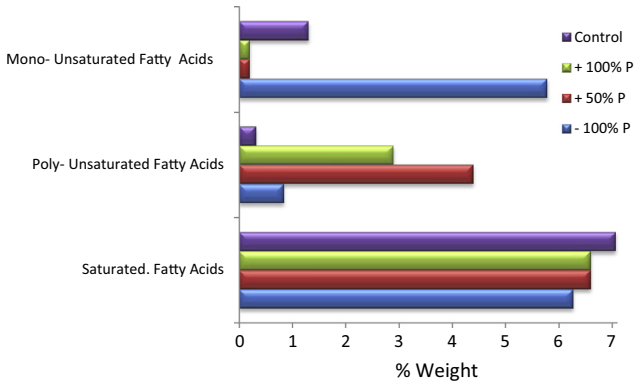

Figure 4 Saturated and unsaturated fatty acid content of *Pichochloruim* Sp. as affected by different concentrations of $NaH_2PO_4{\cdot}2H_2O$ during 12 days of incubation.

culture, lower concentrations of Lauric Acid (C12:0), Tridecanoic Acid (C13:0), Myristoleic Acid (C14:1), Myristic Acid (C14:0), cis-10-Pentadecenoic Acid (C15:1), Pentadecanoic Acid (C15:0), Palmitoleic Acid (C16:1) and Erucic Acid (C22:1) where it recorded 0.49%, 1.82%, 1.02%, 1.68%, 1.13%, 0.96%, 1.38% and 0.33%, respectively.

Discussion

Nitrogen and phosphorus are two important macronutrients for growth and metabolism of algal cells. Nitrogen is an essential constituent of all structural and functional proteins in the algal cells. Phosphorus has a key role in the conveyance of metabolic energy and as an essential structural component of nucleotides and phospholipid molecules in all living cells (Tillberg and Rowley, 1989). It has been widely reported that cell growth of microalgae is affected by either limitation or starvation of essential nutrients (Pinto et al., 2003). Significant differences in the growth of microalgae cells have been observed depending on the limiting nutrient (Ip and Chen, 2005). Similar to the results reported from this study, the cell counts and biomass productivity of the tested alga decreased when grown under nitrogen or phosphorus stress as compared by control culture after 12 days. The limited growth of the alga under (−100%) of nitrogen or phosphorus may be attributed to the presence of nitrogenous as well as phosphorus compounds in the sea water used in the preparation of culture medium for the algal growth. Up to the current knowledge, this is the first report on monitoring the growth of *Pichochloruim* Sp. under nutrient stress.

Considerable variation in the biochemical composition under conditions of nutrient limitation can be observed in algae depending upon which nutrient is limited and to what degree. In this study, the protein content of the tested algal species showed a remarkable decrease during growing on nitrogen deficient media. However, the carbohydrate contents were surprisingly increased in the algal culture grown under the same conditions. In agreement with our findings, Kilham et al. (1997) and Heraud et al. (2005) reported that nitrogen deficiency greatly reduces proteins and thereby increasing the relative carbohydrate content in algal cells. This, in turn, results in a higher lipid/protein ratio as reported by Converti et al. (2009).

Lipid contents increased in the *Pichochloruim* Sp. grown under nitrogen deficiency. As was observed in this study, Converti et al. (2009) reported that the major effects of nitrogen deficiency in algal culture include the enhanced biosynthesis and accumulation of lipids. The amount of lipids in chlorophyta may be raised up to 45% of dry weight by stress or nutrient starvation. Hu et al. (2008) conducted a study on nitrogen stress responses of different microalgae species including green microalgae, all tested species showed a significant rise in lipid production. They concluded that the increase of lipid concentration in stress exposed and ageing cells refers mainly to neutral lipids and triacylglycerols in particular. The observed phenomenon is a result of lipid metabolism shift from membrane lipid synthesis to neutral lipid storage.

This can cause the increase of triacylglycerol (TCG) synthesis *de novo*, as well as the conversion of present membrane lipids to TCG. El-Sheekh et al. (2013) studied optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as a promising microalga for biodiesel production. They concluded that nitrogen deficiency, as a common parameter to increase the lipid content, resulted in a decrease of EFA productivity. Lipid content of freshwater green alga *Chlorella vulgaris* could be significantly increased by 40% in low nitrogen containing medium (Illman et al., 2000). Kilham et al. (1997) revealed that similar to the effects of nitrogen deficiency, phosphorus starvation reduces chlorophyll *a* and protein content thereby increasing the relative carbohydrate content in algal cells. El-Sheekh and Rady (1995) studied the effect of phosphorus starvation on growth, photosynthesis and some metabolic processes in the unicellular green alga *Chlorella kessleri*. They suggested that chlorophyll content decreased during the incubation period of phosphorus starvation whereas there was an increase in the dry weight production which is pronounced when the phosphorus reached a minimum at the end of incubation. From the results of this study it can be observed growth of algal cultures under phosphorous limitation, induced lower proteins and chlorophyll but more carbohydrates.

Phosphorus limitation was found to increase the overall lipid production. As was observed in the results of the present study, Dahmen et al. (2013) on their study on *Picochlorum* Sp. reported that the highest lipid contents were achieved under phosphate starvation and sodium carbonate supplementation, respectively. Under these conditions, the fatty acid profile is dominated by mono-unsaturated and polyunsaturated acids, and is therefore suitable for aqua-culture feeding. In agreement with these results, Khozin-Goldberg and Cohen (2006) reported that the cellular total lipid content of *Monodus subterraneus* increased, mainly due to Triacylglyceride (TAG) accumulation. The green alga *Scenedesmus* Sp. subjected to nitrogen or phosphorus limitation showed an increase in lipids (Xin et al., 2010). Under nutrient limitation; lipid synthesis would be oriented towards the storage of relatively saturated triglycerides, as reported by Piorreck et al. (1984). They stated that nitrogen – as well as phosphorus – participates in the elaboration of phospholipids. In studies carried out on other organisms, including higher plants, the authors have also acknowledged replacement of membrane phospholipids by non-phosphorus containing glycolipids and betain lipids under phosphate limitation (Härtel et al., 2000; Andersson et al., 2003).

When algal growth (as measured by cell divisions) slows down and there is no requirement for the synthesis of new membrane compounds, the cells instead divert and deposit fatty acids into TAG. Under these conditions, TAG production might serve as a protective mechanism. Under normal growth conditions, ATP and NADPH produced by photosynthesis are consumed by generating biomass, with ADP and NADP+ eventually being available again as acceptor molecules in photosynthesis. When cell growth and proliferation are impaired due to the lack of nutrients, the pool of the major electron acceptor for photosynthesis, NADP+, can become depleted. Since photosynthesis is mainly controlled by the abundance of light, and cannot be shut down completely, this can lead to a potentially dangerous situation for the cell, damaging cell components. NADPH is consumed in FA biosynthesis; therefore, increased FAsTAG production might serve as productions (which in turn are stored in TAGs) replenishes the pool of NADP+ under growth-limiting conditions (Thompson, 1996; Hu et al., 2008). On the other hand, Francisco et al. (2010) reported that growth and fatty acid content are inversely related. Phosphorus and nitrogen limitation in this study leads to a decrease in PUFA of microalgal lipids, that is in accordance with the study of Ackman (1982). Similarly, El-Sheekh and Rady (1995) indicated that changes in the proportion of fatty acid composition depend on the growth medium and Pi-starved cells contained much higher concentrations of unsaturated fatty acids than nutrient sufficient algae cells. On the other hand the lipid composition has considerable influence on the technology of biodiesel production and product quality (Li et al., 2013). The unsaturation of the FAME profile is crucial for the overall performance of the final produced biofuel. For instance, biodiesel is mainly constituted of SFA and MUFA, since PUFA decrease the final stability of biodiesel (Demirbas, 2009). Lipids derived from algae cultured without stress contain significant amounts of polar lipids (phospholipids and glicolipids) and limited content of triacylglycerols (TCG), (up to 40% of total lipids) (Harwood and Guschina, 2009; Wang and Wang, 2012). The best material for biodiesel production is TCG while polar lipids are unfavourable since they are a cause of emulsification and catalyst depletion. Lipids other than TCG may also reduce the fuel quality by increasing the content of sulphur and phosphorus (Mendow et al., 2011). Despite reduced growth rate and total lipid production rate, starvation of algae may be beneficial due to the increased content of TCG. Nutrient starvation is one of the most widely used and applied lipid induction techniques in microalgae TAG production and has been reported for many species.

Conclusions and future directions

Growth of *Pichochlorum* Sp. in nutrient deficient medium containing either $NaNO_3$ or $NaH_2PO_4{\cdot}2H_2O$ led to a considerable increase in carbohydrate content. However, the proteins showed a remarkable decrease under the same conditions. A yellowish colour was recorded in the culture under the same conditions indicating the negative effect on chlorophyll. The lipids increased in the cultures growing on the media containing (−50% & −100%) of $NaNO_3$ & $NaH_2PO_4{\cdot}2H_2O$, respectively. Fatty acid methyl ester (FAME) was also found to be improved in algal cultures grown under nitrogen & phosphorus stress (−50% & −100%). Under ($NaH_2PO_4{\cdot}2H_2O$) stress, the fatty acid profile is dominated by mono-unsaturated and polyunsaturated acids, and therefore *Pichochlorum* Sp. can be considered suitable for aqua-culture feeding. However, under ($NaNO_3$) stress, the fatty acids are saturate, and *Pichochlorum* Sp. can be considered is therefore suitable candidate for biodiesel production. Our results suggest that nitrogen and phosphate limitation could be applied to enhance lipid and carbohydrates production.

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